

CLAIMS:

- 5 1. A process for producing Cytotoxic Lymphocyte
Maturation Factor (CMLF) in a substantially pure form comprising:
- stimulating lymphoblastoid cells to produce and secrete
cytokines into a supernatant liquid;
- 10 collecting the supernatant liquid produced by the
stimulated cells;
- separating the supernatant liquid into protein fractions;
- 15 testing each protein fraction for the presence of CLMF by
means of a suitable assay;
- retaining the protein fractions which contain CLMF;
- 20 isolating the CLMF from said CLMF-containing protein
fraction into a substantially pure form.
2. The process of Claim 1 wherein the cells are NC-37 B
- 25 lymphoblastoid cells.
3. The process of Claim 1 wherein the supernatant liquid is
separated into protein fractions by means of a strong cation exchange
column.
- 30 4. The process of Claim 3 wherein the strong cation
exchange is a sulfopropyl cation exchange.
5. The process of Claim 3, further comprising passing the
- 35 protein fractions containing CLMF through an agarose gel, said
agarose gel being comprised of a Blue B dye covalently coupled to an

agarose matrix; and testing the protein fractions which are eluted through said agarose gel for the presence of CLMF and retaining the protein fractions which contain CLMF.

5 6. The process of Claim 5 further comprising eluting the protein fractions containing CLMF of Claim 7 through a strong anion exchange in a High Performance Liquid Chromatography or a Fast Protein Liquid Chromatography mode to obtain protein fractions;

10 testing the protein fractions eluted through the strong anion exchange for the presence of CLMF and retaining the protein fraction containing CLMF.

15 7. A protein comprising Cytotoxic Lymphocyte Maturation Factor (CLMF) in a substantially pure form or a protein which exhibits CLMF activity and contains a biologically active portion of the amino acid sequence of CLMF or which contains an amino acid sequence of CLMF as well as other amino acids or proteins containing analogous sequences to CLMF or its biologically active fragments
20 which proteins exhibit CLMF activity.

8. The protein of Claim 7, wherein CLMF is comprised of a 75 kDa polypeptide, or biologically active fragment thereof.

9. The protein of Claim 8, wherein the 75 kDa polypeptide has an amino acid composition comprised of:

5	<u>Amino acid</u>	<u>mol %</u>
	Aspartic acid or asparagine	10.8
	Threonine	7.2
	Serine	8.9
10	Glutamic acid or glutamine	13.1
	Proline	3.8
	Glycine	4.7
	Alanine	5.9
	Cysteine	2.9
15	Valine	6.2
	Methionine	1.9
	Isoleucine	4.2
	Leucine	9.4
	Tyrosine	3.6
20	Phenylalanine	3.7
	Histidine	1.8
	Lysine	7.7
	Arginine	4.4
25	Tryptophan	ND

10. The protein of Claim 9, wherein the 75 kDa polypeptide is comprised of two disulfide linked subunits, a 35 kDa subunit and a 40 kDa subunit.

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11. The protein of Claim 10, wherein the 35 kDa subunit polypeptide has the following amino acid sequence.

Arg Asn Leu Pro Val Ala Thr Pro Asp Pro Gly MET Phe Pro Cys Leu
5 His His Ser Gln Asn Leu Leu Arg Ala Val Ser Asn MET Leu Gln Lys
Ala Arg Gln Thr Leu Glu Phe Tyr Pro Cys Thr Ser Glu Glu Ile Asp
His Glu Asp Ile Thr Lys Asp Lys Thr Ser Thr Val Glu Ala Cys Leu
Pro Leu Glu Leu Thr Lys Asn Glu Ser Cys Leu Asn Ser Arg Glu Thr
Ser Phe Ile Thr Asn Gly Ser Cys Leu Ala Ser Arg Lys Thr Ser Phe
10 MET MET Ala Leu Cys Leu Ser Ser Ile Tyr Glu Asp Leu Lys MET Tyr
Gln Val Glu Phe Lys Thr MET Asn Ala Lys Leu Leu MET Asp Pro Lys
Arg Gln Ile Phe Leu Asp Gln Asn MET Leu Ala Val Ile Asp Glu Leu
MET Gln Ala Leu Asn Phe Asn Ser Glu Thr Val Pro Gln Lys Ser Ser
Leu Glu Glu Pro Asp Phe Tyr Lys Thr Lys Ile Lys Leu Cys Ile Leu
15 Leu His Ala Phe Arg Ile Arg Ala Val Thr Ile Asp Arg Val Thr Ser
Tyr Leu Asn Ala Ser

12. The protein of Claim 10, wherein the 40 kDa subunit polypeptide has the following amino acid sequence:

20 Ile Trp Glu Leu Lys Lys Asp Val Tyr Val Val Glu Leu Asp Trp Tyr Pro Asp
Ala Pro Gly Glu MET Val Val Leu Thr Cys Asp Thr Pro Glu Glu Asp Gly Ile Thr Trp
Thr Leu Asp Gln Ser Ser Glu Val Leu Gly Ser Gly Lys Thr Leu Thr Ile Gln Val Lys
Glu Phe Gly Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Val Leu Ser His Ser
25 Leu Leu Leu Leu His Lys Lys Glu Asp Gly Ile Trp Ser Thr Asp Ile Leu Lys Asp Gln
Lys Glu Pro Lys Asn Lys Thr Phe Leu Arg Cys Glu Ala Lys Asn Tyr Ser Gly Arg Phe
Thr Cys Trp Trp Leu Thr Thr Ile Ser Thr Asp Leu Thr Phe Ser Val Lys Ser Ser Arg
Gly Ser Ser Asp Pro Gln Gly Val Thr Cys Gly Ala Ala Thr Leu Ser Ala Glu Arg Val
Arg Gly Asp Asn Lys Glu Tyr Glu Tyr Ser Val Glu Cys Gln Glu Asp Ser Ala Cys Pro
30 Ala Ala Glu Glu Ser Leu Pro Ile Glu Val MET Val Asp Ala Val His Lys Leu Lys Tyr
Glu Asn Tyr Thr Ser Ser Phe Phe Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn
Leu Gln Leu Lys Pro Leu Lys Asn Ser Arg Gln Val Glu Val Ser Trp Glu Tyr Pro Asp
Thr Trp Ser Thr Pro His Ser Tyr Phe Ser Leu Thr Phe Cys Val Gln Val Gln Gly Lys
Ser Lys Arg Glu Lys Lys Asp Arg Val Phe Thr Asp Lys Thr Ser Ala Thr Val Ile Cys
35 Arg Lys Asn Ala Ser Ile Ser Val Arg Ala Gln Asp Arg Tyr Tyr Ser Ser Ser Trp Ser
Glu Trp Ala Ser Val Pro Cys Ser

13. The protein of Claim 8 wherein said protein is a biologically active fragment.

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14. An isolated polynucleotide encoding for CLMF or a biologically active fragment thereof.

5 15. A recombinant vector containing the polynucleotide of Claim 14.

16. A host cell transformed with the vector of Claim 15.

10 17. An isolated polynucleotide encoding for a 40 kDa subunit of CLMF having the following DNA sequence:

ATG TGT CAC CAG CAG TTG GTC ATC TCT TGG TTT TOC CTG GTT TTT CTG GCA TCT CCC CTC
15 GTG GCC ATA TGG GAA CTG AAG AAA GAT GTT TAT GTC GTA GAA TTG GAT TGG TAT CCG GAT
GCC OCT GGA GAA ATG GTG GTC CTC ACC TGT GAC ACC CCT GAA GAA GAT GGT ATC ACC TGG
ACC TTG GAC CAG AGC AGT GAG GTC TTA GGC TCT GGC AAA ACC CTG ACC ATC CAA GTC AAA
GAG TTT GGA GAT GCT GGC CAG TAC ACC TGT CAC AAA GGA GGC GAG GTT CTA AGC CAT TCG
CTC CTG CTG CTT CAC AAA AAG GAA GAT GGA ATT TGG TCC ACT GAT ATT TTA AAG GAC CAG
20 AAA GAA CCC AAA AAT AAG ACC TTT CTA AGA TGC GAG GCC AAG AAT TAT TCT GGA CGT TTC
ACC TGC TGG TGG CTG ACG ACA ATC AGT ACT GAT TTG ACA TTC AGT GTC AAA AGC AGC AGA
GGC TCT TCT GAC CCC CAA GGG GTG ACG TGC GGA GCT GCT ACA CTC TCT GCA GAG AGA GTC
AGA GGG GAC AAC AAG GAG TAT CAG TAC TCA GTG GAG TGC CAG GAG GAC AGT GCC TGC CCA
GCT GCT GAG GAG AGT CTG CCC ATT GAG GTC ATG GTG GAT GGC GTT CAC AAG CTC AAG TAT
25 GAA AAC TAC ACC AGC AGC TTC TTC ATC AGG GAC ATC ATC AAA CCT GAC CCA CCC AAG AAC
TTG CAG CTG AAG CCA TTA AAG AAT TCT OGG CAG GTG GAG GTC AGC TGG GAG TAC CCT GAC
AOC TGG AGT ACT CCA CAT TCC TAC TTC TCC CTG ACA TTC TGC GTT CAG GTC CAG GGC AAG
AGC AAG AGA GAA AAG AAA GAT AGA GTC TTC ACG GAC AAG ACC TCA GCC ACG GTC ATC TGC
OGC AAA AAT GCC AGC ATT AGC GTG OGG GOC CAG GAC OGC TAC TAT AGC TCA TCT TGG AGC
30 GAA TGG GCA TCT GTG CCC TGC AGT

18. A recombinant vector containing the polynucleotide of claim 17 or fragment thereof.

35 19. A host cell transformed with the vector of claim 18.

20. An isolated polynucleotide encoding for a 35 kDa subunit polypeptide of CLMF having the following DNA sequence:

5 ATG TGT CCA GCG CGC AGC CTC CTC CTT GTG GCT ACC CTG GTC CTC CTG
GAC CAC CTC AGT TTG GCC AGA AAC CTC CCC GTG GCC ACT CCA GAC CCA
GGA ATG TTC CCA TCC CTT CAC CAC TCC CAA AAC CTG CTG AGG GGC GTC
AGC AAC ATG CTC CAG AAG GCC AGA CAA ACT CTA GAA TTT TAC CCT TGC
ACT TCT GAA GAG ATT GAT CAT GAA GAT ATC ACA AAA GAT AAA ACC AGC
10 ACA GTG GAG GCC TGT TTA CCA TTG GAA TTA ACC AAG AAT GAG AGT TGC
CTA AAT TCC AGA GAG ACC TCT TTC ATA ACT AAT GGG AGT TGC CTG GCC
TCC AGA AAG ACC TCT TTT ATG ATG GCC CTG TGC CTT AGT AGT ATT TAT
GAA GAC TTG AAG ATG TAC CAG GTG GAG TTC AAG ACC ATG AAT GCA AAG
CTT CTG ATG GAT CCT AAG AGG CAG ATC TTT CTA GAT CAA AAC ATG CTG
15 GCA GTT ATT GAT GAG CTG ATG CAG GCC CTG AAT TTC AAC AGT GAG ACT
GTG CCA CAA AAA TCC TCC CTT GAA GAA CCG GAT TTT TAT AAA ACT AAA
ATC AAG CTC TGC ATA CTT CTT CAT GCT TTC AGA ATT CCG GCA GTG ACT
ATT GAC AGA GTG ACG AGC TAT CTG AAT GCT TCC

20 21. A recombinant vector containing the isolated polynucleotide of claim 20.

22. A host cell transformed with the vector of claim 21.

25 23. A method for stimulating LAK cells comprising:

30 treating the LAK cells with CLMF or a biologically active fragment of CLMF and with IL2 or a biologically active fragment thereof.

24. A method for stimulating activated T-cells comprising:
35 treating T-cells with CLMF or a biologically active fragment of CLMF.

25. A method for stimulating activated T-cells as recital in Claim 24 further comprising:

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treating the activated T-cells with IL2 or a biologically active fragment thereof.

26. A method for stimulating Natural Killer Cells comprising:

treating the Natural Killer Cells with CLMF or a biologically active fragment of CLMF.

27. Antibodies in a substantially pure form to CLMF.

28. Antibodies to CLMF of Claim 27 wherein the antibodies are polyclonal.

29. Antibodies to CLMF of Claim 27 wherein the antibodies are monoclonal.

30. A process for producing Cytotoxic Lymphocyte Maturation Factor (CMLF) in a substantially pure form comprising:

stimulating cells capable of producing CLMF to produce and secrete cytokines into a supernatant liquid;

collecting the supernatant liquid produced by the stimulated cells;

separating the supernatant liquid into protein fractions;

testing each protein fraction for the presence of CLMF by means of a suitable assay;

retaining the protein fractions which contain CLMF;

isolating the CLMF from said CLMF-containing protein fraction into a substantially pure form.

31. A process of producing substantially pure CLMF from supernatant liquids obtained from culturing cells, which liquid contains CLMF together with other proteins comprising:

5 separating the supernatant liquid into protein fractions;

testing each protein fraction for the presence of CLMF by means of a suitable assay;

10 retaining the protein fractions which contain CLMF;

isolating the CLMF from said CLMF-containing protein fraction into a substantially pure form.

15 32. A process for producing substantially pure CLMF for liquids containing CLMF together with other proteins comprising:

separating the supernatant liquid into protein fractions;

20 testing each protein fraction for the presence of CLMF by means of a suitable assay;

retaining the protein fractions which contain CLMF;

25 isolating the CLMF from said CLMF-containing protein fraction into a substantially pure form.

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